Influence of Elevated Carbon Dioxide on Interactions Between *Frankliniella occidentalis* and *Trifolium repens*

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ABSTRACT Elevated CO_2 concentrations can increase plant growth and change plant nutritive value for herbivores. Several reports indicate that leaf-chewing insects consume more foliage of plants grown at elevated CO_2 concentrations than of plants grown at ambient CO_2. Research with additional plant–pest systems is needed to determine if this phenomenon is widespread and if increased insect feeding might affect productivity. Effects of CO_2 enrichment on foliar consumption and population size of Western flower thrips [*Frankliniella occidentalis* (Pergande)]) were measured on white clover (*Trifolium repens* L.). White clover infested with thrips was exposed for 24 h/d to ≈396 (ambient) or 745 μLL^{-1} (elevated) CO_2 for up to 35 d in 10 greenhouse exposure chambers. At elevated CO_2, clover shoot weight and laminae weight were ≈50% greater, and laminar area was ≈20% greater than at ambient CO_2. Thrips population size was not significantly affected by CO_2, but laminar area scarred by thrips feeding was ≈90% greater at elevated than at ambient CO_2. Because of increased growth, however, undamaged leaf area was approximately15% greater at elevated than at ambient CO_2.

KEY WORDS *Trifolium repens*, white clover, carbon dioxide enrichment, *Frankliniella occidentalis*, Western flower thrips

INCREASED ATMOSPHERIC CARBON DIOXIDE (CO_2) affects plant photosynthesis and chemistry (Rogers et al. 1983a, Cure and Aycock 1986, Kimball 1986), thereby influencing plant tissue nutritive quantity and quality for arthropods. Leaves of green plants at elevated CO_2 generally contain higher percentage soluble carbohydrates and lower percentage N than those at ambient CO_2 (Watt et al. 1995, Bezemer and Jones 1998, Coviella and Trumble 1999). Leaf chewing insects often consume more foliage of plants grown in CO_2-enriched air than of plants grown at ambient CO_2, possibly to compensate for decreased foliar N (Lincoln et al. 1986, Weste et al. 1987, Lincoln 1993, Watt et al. 1995, Brooks and Whittaker 1998, Buse et al. 1998, Lindroth and Kinney 1998, Stiling et al. 1999). The few studies of CO_2 enrichment effects on populations of whole-cell feeding arthropods have provided mixed results. Carbon dioxide enrichment suppressed populations of greenhouse whiteflies [(*Trialeurodes vaporariorum* (Westward)] on tomato (Tripp et al. 1992) but increased populations of the twospotted spider mite (*Tetranychus urticae* Koch) on white clover (Heagle et al. 1994a, 2002). Populations of sweet potato whitefly [(*Bemesia tabaci* (Gennadius)] on cotton (Butler et al. 1986) and of Western flower thrips [(*Frankliniella occidentallis* (Pergande)] on milkweed (*Asclepias syriaca* L.) (Hughes and Bazzaz 1997) were not affected by CO_2 enrichment. Thrips (probably *F. occidentalis*) populations in cotton *Gossipium hirsutum* L. also were not significantly affected by CO_2 enrichment of their cotton host (Butler 1985)

Western flower thrips (*F. occidentalis*) is one of the most important and difficult to control plant pests. It feeds on numerous plant species and spreads the tomato spotted wilt virus, which also affects numerous plant species. One experiment showed that consumption of *A. syriaca* leaves by *F. occidentalis* was significantly greater on plants exposed to elevated CO_2 compared with ambient levels, although the population size was not significantly affected (Hughes and Bazzaz 1997).

During recent greenhouse experiments, a severe infestation of *F. occidentalis* precluded further work to determine effects of mixtures of O_3 and CO_2 on white clover (*Trifolium repens L.*). This infestation provided an opportunity to study the effects of CO_2 enrichment on interactions between *F. occidentalis* and *T. repens.* Our objective was to determine if CO_2 enrichment affects foliar consumption and population size of *F. occidentalis* on *T. repens.*

Materials and Methods

General. The experiment was performed in a non-filtered-air greenhouse 5 km south of Raleigh, NC,

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Table 1. Carbon dioxide conenctrations, temperature, relative humidity (RH), and PAR during exposures of white clover and thrips to carbon dioxide[a]

Trial	Dates of exposure	Carbon dioxide concentration (μLL^{-1})		Temperature (°C)	RH (%)	PAR (mol m^2/d)
		Ambient	Double ambient			
1	27 Feb.–26 Mar	393	729	20	58	34
2	3 Apr.–7 May	398	761	22	62	30

[a] All values are 24-h means. Values for CO_2 concentrations across replicate chambers are within 3% of values shown. Values for temperature across replicate chambers were within 1°C of values shown. Values for RH across replicate chambers were within 1% of values shown. Values for PAR across chambers were within 9% of values shown.

between late February and early May. A *T. repens* clone (NC-S) previously described (Heagle et al. 1993, 1994b) was propagated by rooting virus-free stolons in 1-liter pots containing Metro Mix 220 and 4 g of slow release Ozmocote fertilizer (14–14-14, N-P-K; Scotts-Sierra Horticultural Products, Marysville, OH). Plants were inoculated with commercial rhizobium inoculant 14 d after start of rooting and were watered as needed to prevent wilting throughout the experiment.

Exposures. Plants were exposed to ambient and approximately double ambient CO_2 concentrations in 10 cylindrical (1.07 m diameter by 1.20 m tall) continuous-stirred tank reactor chambers (CSTRs) continuously aspirated with charcoal-filtered air (Heck et al. 1978). Ambient greenhouse light was supplemented by 1,000-W multivapor lamps that supplied $\approx$270 μmol/m^2/s of photosynthetically active radiation (PAR) to each chamber from 0600 to 1800 h EST daily. Temperature was monitored in the CSTRs with copper-constantan thermocouples, PAR was measured with quantum sensors (LI-190S-1; LI-COR, Lincoln, NE), and relative humidity was measured with Vaisala HMP 31UT sensors (Vaisala, Woburn, MA).

A naturally occurring population of *F. occidentalis* was present on *T. repens* growing in 15-liter pots. New plants were rooted as described above in 1-liter pots on a bench between two benches that each contained ten 15-liter pots of the thrips-infested plants. Evidence of thrips injury on new plants appeared as irregular white necrotic areas and distortion of newly expanding trifoliolates within 3 wk after rooting began.

The experimental design was a randomized complete block with five blocks of the two CO_2 concentrations as the main plot (CSTR) treatments with nine 1-liter pots of clover randomly assigned to each of the 10 CSTRs. Plants were moved to the CSTRs 26 d after rooting began for trial 1 and 21 d after rooting began for trial 2. Exposures to CO_2 for 24 h/d began 1 d after plants were moved to the CSTRs. CO_2 was obtained from tank CO_2 and was monitored sequentially in each chamber with an infrared CO_2 analyzer (LI 6252 (LI-COR). Details of CO_2 dispensing and monitoring protocols used in this study have been described (Rogers et al. 1983b). Exposures continued for 27 d for trial 1 and for 35 d for trial 2. Mean concentrations of CO_2, temperature, PAR, and relative humidity during CO_2 exposure for each trial are shown in Table 1.

Measurements. Six plants in each CSTR were used to measure thrips populations, and three plants in each chamber were used to measure area and weight of leaf laminae and to estimate foliar scarring by thrips. To measure thrips populations, we modified a procedure for collecting thrips on fresh pole bean pods previously described (Groves et al. 2001). One day before exposures ended, foliage and stems from three two-plant samples per chamber were cut and placed in white plastic containers (20 cm diameter by 16 cm tall). To ensure air exchange, organdy cloth was used to replace a 10-cm-diameter area of the bottom and the entire top of each container. One fresh pole bean pod (*Phaseolus vulgaris* L.) was placed at the bottom of each container as a food and moisture source for the thrips. The containers were placed on their sides in a desiccation chamber set at 28°C and 20% RH. For trial 1, the clover foliage was dry after 3 d. Thrips still among the dried foliage in each container were encouraged to move downward toward the bean pod by exhaling into the container while gently rustling and slowly removing the dried foliage. Bean pods with thrips were placed in ethyl alcohol (96%), and adults and immature thrips were counted later using a microscope. The dried foliage for each two-plant sample was weighed. For trial 2, the procedure was similar except drying required 7 d because plants were larger than for trial 1. For trial 2, plant material from each container (<1 g) remaining after bean pods were collected was placed in plastic bags. Adults and immature thrips in this sample and in ETOH were counted with a microscope.

Within 3 h after exposures ended, area of leaf laminae was measured for three plants per chamber using an electronic meter (LI-3100; LI-COR). Thrips feeding on expanded leaves caused irregular pale scarred areas on abaxial leaf surfaces. The percentage scarred area was visually estimated for each leaflet (0–100%). Total leaf area scarred per plant was calculated as mean area per leaflet $\times$ mean percentage area per leaflet scarred by thrips $\times$ number of leaflets per plant. Leaf laminae were dried at 50°C and weighed.

Statistical Analyses. Analyses of variance (ANOVA) were performed on chamber means. Analysis of the trials separately showed similar responses to treatments. Data from both trials were then combined and analyzed with trial considered to be a fixed factor. Residual plots were examined for non-normality, outliers, and heterogeneous variances. The Box-Cox test indicated that percentage leaf area scarred by thrips was best analyzed using the log transformation. All other variables were analyzed using original scales.

Table 2. Effects of CO_2 enrichment on *T. repens* growth and foliar consumption of *T. repens* by *F. occidentalis*[a]

Trial	CO_2 concentration μLL^{-1}	Shoot dry weight per 2-plant sample (SE)[a] (g)	Laminae wt per plant (SE) (g)	Number of leaflets per plant (SE)	Laminar area per plant (SE) (cm^2)	Thrips per 2-plant sample[a]: immatures (SE)	Thrips per 2-plant sample[a]: adults (SE)	Laminar area scarred per plant[b] (cm^2)
1	393	13.5 (0.14)	4.6 (0.11)	127 (1.6)	628 (17.7)	278 (27.9)	21 (1.7)	31 (3.6)
	729	20.1 (1.39)	6.8 (0.23)	127 (1.0)	748 (25.9)	270 (36.7)	13 (0.9)	60 (4.4)
2	398	17.6 (0.33)	6.5 (0.23)	170 (2.0)	1017 (39.6)	533 (060.2)	27 (3.0)	73 (15.0)
	761	27.1 (1.02)	10.3 (0.28)	187 (1.2)	1232 (19.1)	516 (143.5)	32 (7.6)	139 (29.6)
Source	df	Probability of $>F$ value from ANOVA						
Trial	1	0.0003	0.0001	0.0001	0.0001	0.0249	0.0092	0.0430
CO_2	1	0.0001	0.0001	0.0005	0.0001	0.8630	0.7854	0.0007
Trial × CO_2	1	0.1326	0.0111	0.0007	0.1165	0.9512	0.2092	0.8919

[a] Each value is the mean of 15 samples (three two-plant samples or three one-plant samples in each of five replicate chambers).
[b] Laminar area scarred per plant calculated as (mean area per leaflet × mean percentage area scarred per leaflet × number of leaflets).

Results

Plants were larger in trial 2 than trial 1, and the trial effect was significant for all responses measured (Table 2). Plants at elevated CO_2 were larger than at ambient CO_2, and the CO_2 effect was significant for all plant responses measured. Shoot weight and leaf lamina weight were ≈50% greater in elevated than at ambient CO_2 in both trials. Leaf numbers were not affected by CO_2 in trial 1 but were greater at elevated than at ambient CO_2 in trial 2, accounting for the significant trial × CO_2 effect. Laminar area was ≈20% greater at elevated than at ambient CO_2 in both trials.

Thrips populations were ≈1.3 times greater in trial 2 than in trial 1, but population size was not significantly affected by CO_2 in either trial (Table 2). However, laminar area per plant scarred by thrips feeding was ≈90% greater in elevated than ambient CO_2 in both trials (Table 2). In trial 1, per capita leaf area scarred (laminar area scarred per plant divided by total thrips population per plant) averaged 0.21 cm^2 at ambient CO_2 and 0.42 cm^2 at elevated CO_2. Comparable values for trial 2 were 0.26 and 0.51 cm^2, respectively.

Discussion

The current study essentially corroborates previous results showing increased per capita feeding of milkweed by Western flower thrips with no significant thrips population change (Hughes and Bazzaz 1997). Neither the present or previous results should be considered proof that elevated CO_2 does not affect thrips populations, however. A major reason is that neither study simulated seasonal exposure duration. Exposures of clover lasted 27 d in trial 1 and 35 d in trial 2 at temperatures averaging 20–22°C. Exposure of milkweed was ≈28 d (Hughes and Bazzaz 1997). These durations allowed time for only an estimated 1.5–2.0 generations (Martin 1993, Baker 1994).

Nutritive changes may be partly responsible for effects of CO_2 enrichment on herbivores, but evidence is mostly limited to correlations between insect response and whole-leaf analyses showing increased C, C/N ratios, and carbohydrates accompanied by decreased N. Foliar nutritive levels were not measured in the present experiment. However, previous analyses of leaves of *T. repens* (NC-S) showed that elevated CO_2 decreased percentage N by 13%, increased nonstructural carbohydrates by 49%, and did not significantly affect the concentration of any of 14 amino acids (Heagle et al. 2002).

Most research measuring effects of elevated CO_2 has included only two CO_2 concentrations (ambient and double ambient). It is not possible to accurately estimate effects of intermediate CO_2 concentrations from studies using only two concentrations because responses to CO_2 may or may not be linear. Because ambient CO_2 concentrations have been rising over the past century, the two-concentration approach does little to show if increases in atmospheric CO_2 that have already occurred are causing significant effects on plants or plant pests. Dose–response designs with multiple CO_2 concentrations are needed to develop models that can estimate effects at all CO_2 concentrations.

Whereas consumption of white clover by thrips at elevated CO_2 was ≈90% greater than that at ambient CO_2, total leaf area increased by ≈20%, and undamaged leaf area increased by ≈15%. This net increase in undamaged leaf area occurred despite feeding by a relatively high thrips population. Whether or not elevated CO_2 will be a net benefit for *T. repens* under field conditions remains to be determined.

The present results show that increased feeding by *F. occidentalis* in response to CO_2 enrichment is not limited to a given plant species. Because both *F. occidentalis* and tomato spotted wilt virus have an extremely wide host range, the possibility that elevated CO_2 might increase the spread and prevalence of tomato spotted wilt virus should be investigated.

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